Package Insert

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QuantiGene Sample Processing Kit

FFPE Tissues

About Sample Processing Kits

Sample Processing Kits are designed for use with both single plex and multiplex QuantiGene assays for quantification of RNA or DNA targets directly from a variety of sample types.

About this Kit

This QuantiGene Sample Processing Kit for FFPE Tissue Homogenates contains reagents and instructions for the preparation of tissue homogenates from FFPE tissue sections for use in QuantiGene 2.0 and QuantiGene Plex 2.0 assays for RNA targets and QuantiGene Plex DNA assays for DNA targets. For more information, refer to the appropriate QuantiGene Reagent System User Manual.

! **IMPORTANT: For quantitating RNA targets, we highly recommend the use of QuantiGene 2.0 Sample Assessment Kit to evaluate relative cell number and RNA quality of FFPE tissue homogenates. For more information, see the *QuantiGene 2.0 Sample Assessment Kit Package Insert*.**

Contents and Storage

Kit components have a shelf life of 12 months from the date of delivery.

Table 1 Kit contents and storage conditions

Cat. No.	QS0107	QS0108	QS0109	
Kit Size	**10 Samples***	**25 Samples***	**100 Samples***	**Storage**
Component	**Quantity**	**Quantity**	**Quantity**	
Homogenizing Solution	10 mL	20 mL	75 mL	15-30 °C
Proteinase K† (50 µg/µL)	36 µL	90 µL	360 µL	–20 °C

** A sample is defined as 25-100 mm² x 50-60 µm total thickness of FFPE tissue sections.*

† Place on ice during use. We recommend storage at –20 °C in an enzyme storage box, for example NEB Cool Box (New England Biolabs P/N T0400S). NEVER store at –80 °C.

Safety Warnings and Precautions

All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

What this Package Insert Covers

This package insert provides recommendations and step-by-step procedures for the following:

- *Materials Required but not Supplied*
- *Preparing FFPE Tissue Homogenates*
- *Determining Complete Tissue Homogenization*
- *Clarifying Homogenates*
- *Dewaxing of FFPE Samples (Optional)*

Materials Required but not Supplied

Table 2 Required materials not supplied

Item	Source
RNase Zap® (if quantifying RNA)	Ambion P/N AM0780
Disposable razor blades or scalpels	Major laboratory supplier

Preparing FFPE Tissue Homogenates

Before You Start

If you are planning to quantitate RNA targets, treat all surfaces with RNase Zap according to the manufacturer's instructions.

Procedure

To prepare tissue homogenates from FFPE tissue sections:

1. Measure and transfer the tissue.
   For FFPE tissue sections:
   - **A.** Measure the length (L) and width (W) of the tissue and calculate the cross-sectional area (L x W) in square millimeters (mm^2).
   - **B.** Using a clean razor blade or scalpel, scrape the slide to completely remove the FFPE section and transfer it to a 1.5-mL microfuge tube. Avoid transferring excess paraffin.

     IMPORTANT: For best results, ensure adequate sample input by combining the equivalent of 50–60 µm thick x 25-100 mm^3 area of tissue. For example, if sections are 10 µm in thickness, combine 5–6 sections into a single microfuge tube.

     NOTE: H & E stained slides are compatible with QuantiGene and QuantiGene Plex assays.

     NOTE: Optional. See *Dewaxing of FFPE Samples (Optional)* on page 4 for dewaxing of samples.

2. Solubilize the tissue:
   - **A.** Using the volumes specified in the tables below, add Homogenizing Solution and Proteinase K to the tissue.
     For tissue sections (50-60 µm combined total thickness):

Table 3 Tissue input for preparing homogenates

Tissue Area (mm^2)	Homogenizing Solution (µL)	Proteinase K Volume (µL)
25-100	300	3
100-225	600	6
>225	900	9

   - **B.** If quantitating DNA targets, incubate the samples at 65 °C for 30 minutes to 1 hour. Briefly vortex the samples, then return them to 65 °C overnight (16–20 hours).
   - **C.** If quantitating RNA targets, incubate the samples at 65 °C for 6 hours. For every hour of incubation, vortex samples for 1 minute at maximum speed.

3. Centrifuge the samples in a microfuge at maximal speed for 5 minutes at room temperature to pellet the cellular debris, then transfer the homogenate to a fresh microfuge tube, avoiding any residual paraffin and debris. Repeat if necessary to completely remove debris.

NOTE: Any residual paraffin will solidify at room temperature during centrifugation, and may appear as a solid residue above the homogenate. It might be necessary to pierce the solid paraffin layer with a pipette tip in order to transfer the homogenate. Discard the pipette tip if it becomes clogged.

4. Use the homogenate immediately in a QuantiGene or QuantiGene Plex assay or store at –80 °C for future use.

Determining Complete Tissue Homogenization

We strongly recommend you validate your homogenate by doing the following:

- Examine the homogenate. It should be clear and non-viscous.
- Perform a serial dilution of the homogenate and run an appropriate QuantiGene or QuantiGene Plex assay with it. Verify the expected fold change matches the observed fold change. For example, a 3-fold dilution should generate 3-fold changes (± 20%) in the signal (background subtracted) of the targeted genes.

Clarifying Homogenates

When using the QuantiGene Plex assay with Filter Plates, it is very important that all extracellular debris is removed from the cell lysate. Failure to remove particulates might result in clogged wells on the Filter Plate following the overnight hybridization step which could lower assay precision.

NOTE: If using magnetic separation, clarification of samples is rarely necessary.

Required Materials

Table 4 Required materials for clarifying lysates

Item	Source
0.45 µm cellulose nitrate filter plate	Affymetrix, P/N PC5512 or Whatman, P/N 7700-3307
96-well polypropylene plate (collection plate)	Fisher P/N 07-201-156 (Corning 3371)
Adhesive plate seal	Major laboratory supplier
Microplate centrifuge	Eppendorf 5804R and rotor A-2 DWP or equivalent

Procedure

To clarify homogenates:

1. Determine the number of wells to use on the cellulose nitrate filter plate, based on the number of samples and volume prepared for each sample.
   Seal the wells that will not be used with an adhesive plate seal.

IMPORTANT: Do not add more than 300 µL/well.

2. Add the samples to the 0.45 µm cellulose nitrate filter plate.
3. Place cellulose nitrate plate (with samples) on top of the collection plate.
4. Spin the nitrate plate/collection plate assembly in the microplate centrifuge at 1,444 x g for 2–5 minutes at room temperature. If the sample has not filtered through the cellulose plate, spin an additional 2–3 minutes.
5. Use lysates immediately in a QuantiGene or QuantiGene Plex assay, or seal the plate with an adhesive seal and store at –80 °C for later use.

Dewaxing of FFPE Samples (Optional)

Table 5 Required materials for dewaxing

Item	Source
Concentrated Dewaxing Solution*	Affymetrix, P/N QS0508

** Add 100% ethanol before use.*

Procedure

NOTE: Precipitates may form in the De-Waxing Solution. Re-dissolve by incubating at 37 °C followed by gentle swirling.

To dewax FFPE samples:

1. Measure and transfer tissue:
   - **A.** Measure the length (L) and width (W) of the tissue and calculate the cross-sectional area (L x W) in square millimeters (mm2).
   - **B.** Using a clean scalpel, completely scrape the tissue from the slide(s) and transfer it to a microcentrifuge tube. Avoid transferring excess paraffin.
2. De-wax the sample:

   IMPORTANT: Add 100% ethanol, according to the label, to concentrated Dewaxing Solution before use.

   - **A.** Add 1 mL Dewaxing Solution and vortex for 30 seconds. Incubate for 5 minutes at room temperature.
   - **B.** Spin the sample in a microcentrifuge at maximum speed for 5 minutes at room temperature to pellet the tissue.
   - **C.** Without disturbing the pellet, remove the supernatant and discard.
   - **D.** Add 1 mL 70% ethanol and vortex for 30 seconds. Incubate for 2 minutes at room temperature.

     NOTE: Prepare 70% ethanol fresh weekly from 100% ethanol and nuclease-free water.

   - **E.** Spin the sample in a microcentrifuge at maximum speed for 5 minutes at room temperature to pellet the tissue.
   - **F.** Without disturbing the pellet, remove the supernatant and discard.
3. Repeat step 2 one or more times until paraffin is visibly reduced. It is not necessary to completely remove the paraffin.
4. Rinse tissue to remove all traces of ethanol:
   - **A.** Gently add 1 mL nuclease-free water. Do not mix.
   - **B.** Spin in a microcentrifuge at maximum speed for 5 minutes at room temperature to pellet the tissue.
   - **C.** Without disturbing the pellet, completely remove the supernatant and discard.

     NOTE: If rehydrated tissue does not form a compact pellet, increase centrifugation time to 15 minutes.

5. Return to the procedure on page 2 to solubilize the tissue and complete sample preparation.

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